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This work was undertaken because of some findings on experimental lathyrism, namely, that the "carbonyl fixatives", iproniazid and semicarbazide, either aggravate the symptoms of experimental lathyrism (Juva, Mikkonen, Tuominen & Kulonen 1959; Roy, Lipton, Strong & Bird 1959) or cause similar effects when given alone (Neumann, Maxwell & McCoy 1956). Experimental lathyrism is clearly influenced by the amount of amino acids in the diet, without regard to the composition of the amino acid mixture (Juva et al. 1961). Thus the general intermediary metabolism of amino acids, and consequently of keto acids, is involved in β-aminopropionitrile poisoning. We were also interested to find out whether any unusual carbonyl derivates were present in the tissues of lathyritic animals.

Experimental

Chick embryos. The fertilized eggs were a gift from Messrs. Turun Muna Oy, Turku, Finland. After 9 days of hatching, 10 mg of β-aminopropionitrile (as a 10 % aqueous solution of its hydrogensulphate, half the LD50, prepared as described by Ford, Buc & Grenier 1947) were injected into the yolk sacs. The controls received an equimolar amount of sodium sulphate. In some experiments both groups of eggs received additionally 10 mg of semicarbazide hydrochloride (Merck pro analysi) to trap carbonyl compounds. After 4 hours of continued hatching, the embryos were harvested, weighed and homogenized in 10 ml of water. The homogenate was treated as descibed by Richter (1937), and the resulting 2,4-dinitrophenyl-hydrazone mixture chromatographed by Kulonen, Carpén & Ruokolainen's procedure (1952).

Rats. "Wistar" rats received the lathyritic (sweet pea) or control diets described earlier (Juva et al. 1961). To decrease variation four rats were treated as a group, and three groups were on each diet. In the first experiment (average weight of the rats at the beginning 62 g, after 29 days experiment 87 g in the lathyrus group and 112 g in the control group) the cages were placed above large funnels containing a wire net to retain faeces. Sulphuric acid was put beforehand in the receiving beakers to inhibit microbial growth in the urine. In the second experiment

the animals were a little smaller (at the beginning 46 g, after 23 days on the lathyrus diet 63 g and on the control diet 80 g). To collect the urine, the falling faeces were diverted outside the recipient beaker by a glass spindle placed below the funnel, and contamination with faeces was thus avoided. Toluene was added to inhibit infection. For analysis, the urines of 3-4 consecutive days were pooled. The animals were then killed by decapitation, and the blood was collected into weighed centrifuge tubes. The blood and urine samples were deproteinised with tungstic acid, and the supernatants were treated with 2,4-dinitrophenylhydrazine solution (Krusius 1940). The hydrazones of the acid oxo compounds of blood and urine were then prepared and analyzed as indicated above (Kulonen et al. 1952).

Results

The final results are collected in table 1. The statistical significance of differences was calculated by the t-test. The urine concentrations were calculated as daily averages, and simultaneous lathyritic and control samples were compared.

Table 1.

Effect of β-aminopropionitrile on tissue, blood and urine concentrations of α-ketoglutaric and pyruvic acids. Standard deviations and (in the brackets) the number of samples also given. SC semicarbazide, NS difference not significant ($P > 0.05$).

Sample		α-Ketoglutaric acid		
		Control	Treated	P
Chick embryos, µg/g				
without SC		11.9±4.8(6)	14.5±4.6(6)	NS
with SC		16.5±7.0(4)	15.2±3.5(5)	NS
Rat blood, µg/g	exp.1	1.6±1.0(13)	2.0±0.9(12)	NS
	exp.2	-	-	-
Rat urine[1]), µg/24 hr.				
	exp.1	1490±450	460±340	< 0.001
	exp.2	200±150	100±56	< 0.05
		Pyruvic acid		
		Control	Treated	P
Chick embryos, µg/g				
without SC		5.7±1.9(6)	9.1±2.8(6)	< 0.05
with SC		17.4±9.7(4)	25.0±5.5(5)	NS
Rat blood, µg/g	exp.1	4.2±2.0(9)	5.8±2.1(5)	NS
	exp.2	5.0±2.0(11)	5.6±2.3(12)	NS
Rat urine[1]), µg/24 hr.				
	exp.1	67±10	48±15	NS
	exp.2	86±39	58±17	NS

The variations are large, but the results for urine are in satisfactory agreement with the findings of Krusius (1940). From adult rats he obtained an excretion of α-ketoglutaric acid of 0.07 mg/12 hr. on a high fat diet and 0.47 mg/12 hr. on a high carbohydrate diet. For the excretion of pyruvic acid the range on different diets was still larger, 0.03-1.77 mg/12 hr. The blood values are in a reasonable agreement with those for man (Kulonen et al. 1952). We are unable to explain the large difference in α-ketoglutaric excretion between the first and second experiments. In the first the animals were larger, but this does not seem a sufficient explanation. We are rather inclined to believe that the values for the first experiment are unreliable because of contamination of the samples with faeces.

Since the different experiments point in the same direction, we suggest that the concentrations of pyruvic acid, and to a less extent of α-ketoglutaric acid, are increased in lathyrism both in embryonic tissue and in blood, but that their urinary excretion is decreased. Considering the smaller bodyweight of the intoxicated animals, the difference in the excretion is scarcely of metabolic consequence. However, this is not certain, since excretion seems to decrease immediately after the first consumption of the lathyrus diet.

There was no obvious qualitative difference in the keto acids between normal and lathyritic tissues. Some possible intermediates, for example, the 2,4-dinitrophenylhydrazone of the glutamic semi-aldehyde, may have migrated in chromatograms so near to the front that they escaped attention.

Discussion

The best known cause for the increase in keto acids of the tissues is an inhibition of the oxidative decarboxylation because of a shortage of thiamine. Nothing is known about oxidative decarboxylation in lathyritic tissue or in the presence of β-aminopropionitrile. We have conducted some *in vitro*-experiments on the consumption of oxygen in liver slices with pyruvic acid as substrate. A slight decrease was noted in the presence of β-aminopropionitrile, but first only at a concentration of 10^{-2} M. The neutral fat of rat skin is markedly decreased in lathyrism, but it is not known whether the conversion of the pyruvic acid to fats is impaired.

The other possible reason for the increase in keto acids may be disturbed transamination. The amination of pyruvate is depressed by all enzyme inhibitors blocking intermediate steps from pyruvate to ketoglutarate via the citric acid cycle (Braunstein & Azarkh 1957 a). In isoniazid-drugged animals the transamination of the α-keto acids is also depressed (Braunstein & Azarkh 1957 b). *In vitro*-experiments (with T. Nikkari) to reproduce with β-aminopropionitrile the effect of isoniazid on the liver amino-transferase were not successful.

The addition of semicarbazide, which itself has an effect similar to that of lathyrism, increased the concentration of pyruvic acid three-fold, but did not affect the α-ketoglutaric acid. The difference between the control and lathyritic sample also persisted. The results from the experiment with semicarbazide were not accurate enough to permit conclusions about the mechanism of the increase in pyruvic acid, i.e. to decide between increased formation or decreased breakdown.

Summary

The pyruvic and α-ketoglutaric acid concentrations were moderately increased in β-aminopropionitrile-treated chick embryos and in the blood of lathyritic rats.

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